

Species comparison of hepatic and pulmonary metabolism of benzene

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Received 13 May 1999; accepted 4 September 1999

Abstract

Benzene is an occupational hazard and environmental toxicant found in cigarette smoke, gasoline, and the chemical industry. The major health concern associated with benzene exposure is leukemia. Studies using microsomal preparations from human, mouse, rabbit, and rat to determine species differences in the metabolism of benzene to phenol, hydroquinone and catechol, indicate that the rat is most similar, both quantitatively and qualitatively, to the human in pulmonary microsomal metabolism of benzene. With hepatic microsomes, rat is most similar to human in metabolite formation at the two lower concentrations examined (24 and 200 μM), while at the two higher concentrations (700 and 1000 μM) mouse is most similar in phenol formation. In all species, the enzyme system responsible for benzene metabolism approached saturation in hepatic microsomes but not in pulmonary microsomes. In pulmonary microsomes from mouse, rat, and human, phenol appeared to competitively inhibit benzene metabolism resulting in a greater proportion of phenol being converted to hydroquinone when the benzene concentration increased. The opposite effect was seen in hepatic microsomes. These findings support the hypothesis that the lung plays an important role in benzene metabolism, and therefore, toxicity. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Benzene; Lung; Liver; Rat; Mouse; Rabbit; Human

1. Introduction

Benzene is a toxic occupational hazard and environmental pollutant. It is used in manufacturing products such as rubber, lubricants, detergents, drugs, and pesticides. It is also present in gasoline. The most common exposures occur

through auto exhaust, industrial emissions, and cigarette smoke (ASTDR, 1997). Auto exhaust and industrial emissions account for ~20% of human exposure while exposure to cigarette smoke accounts for ~50%. Background levels of benzene in air range from 2.8 to 20 parts per billion (ppb). Estimates indicate that occupational exposure affects as many as 238 000 people (ASTDR, 1997).

Chronic exposure to benzene can result in anemia, thrombocytopenia, leukopenia, and/or aplas-

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tic anemia (Robles, 1998). However, the main health concern for humans exposed to low levels of benzene is acute myelogenous leukemia (Infante et al., 1977; Askoy, 1980, 1981, 1985). There is also limited evidence in humans which indicates that lung cancer may result from benzene exposure (Askoy, 1980, 1981; Yin et al., 1996).

Currently no animal model mimics the leukemogenic effect of benzene in humans. However, inhalation studies have shown an increase in various types of tumors, including Zymbal's gland and lung tumors, in mice (NTP, 1986; Snyder et al., 1988; Farris et al., 1993). An increase in various types of tumors, such as Zymbal's gland, nasal cavity, and oral cavity, has also been demonstrated in rats (NTP, 1986; Maltoni et al., 1989). Snyder et al. (1988) showed that low level, discontinuous exposure over a lifetime has a greater effect, as judged by tumor response in mice, than short term intense exposure.

Benzene metabolism is complex. The proposed first step in benzene metabolism is the transformation of benzene to benzene oxide by the cytochrome P450 multi-functional oxygenase system (Jerina et al., 1968). Both the CYP2E1 and CYP2B1 isozymes may be involved in benzene metabolism. Benzene oxide is non-enzymatically transformed to either phenol (Parke and Williams, 1953) or ring-opened muconaldehyde (Latrino et al., 1986). Muconaldehyde is metabolized to muconic acid (Kirley et al., 1989). Phenol is further metabolized, again via cytochrome P450, to hydroquinone (Koop et al., 1989) and catechol. Hydroquinone and catechol can then be metabolized in bone marrow by myeloperoxidase to form *p*-benzoquinone and *o*-benzoquinone (Sawahata and Neal, 1982; Sadler et al., 1988; Smith et al., 1989; Subrahmanyam et al., 1991).

Detoxification reactions are also important in benzene metabolism. These include glutathione conjugation of benzene oxide, and sulfate and glucuronide conjugation of phenol, hydroquinone, catechol, and trihydroxybenzene (Henderson et al., 1989). Following conjugation, metabolites are excreted in the urine. The most abundant conjugated metabolite found in primates is phenyl sulfate while hydroquinone glucuronide, muconic acid, phenylglucuronide,

hydroquinone sulfate, and catechol sulfate are detected in smaller amounts (Sabourin et al., 1992).

The toxicity of benzene is related to its metabolism. The metabolites thought to be involved are phenol, hydroquinone, catechol, and possibly benzene oxide. As noted above, the first step proposed in benzene metabolism is the formation of benzene oxide. Benzene oxide had not been measured as a metabolite until recently when it was measured in vivo (Lindstrom et al., 1997) as well as in vitro (Lovern et al., 1997). Lindstrom et al. (1997) showed that benzene oxide has a half-life in the blood of rats of ~8 min, which suggests it could travel from the site of metabolism, presumably the liver, to bone marrow. Since benzene oxide is a relatively stable electrophilic compound, it may contribute directly to benzene toxicity (Lovern et al., 1997; Lindstrom et al., 1997).

Another hypothesis suggests that hydroquinone is further metabolized by myeloperoxidase in bone marrow to *p*-benzoquinone which is responsible for the leukemogenic effect of benzene. This highly reactive quinone may react with progenitor cells in bone marrow and cause toxicity in the following ways: free radical formation, covalent binding to DNA, RNA, or other macromolecules, or alkylation of –SH groups (Irons, 1985).

The interaction of multiple metabolites may be implicated. Eastmond et al. (1987) showed that the presence of phenol increased the metabolism of hydroquinone by horseradish peroxidase in vitro by 200%. With purified human myeloperoxidase the increase was 400%. In vivo experiments demonstrated that phenol or hydroquinone alone did not cause significant damage to bone marrow in mice. When administered together, a significant decrease in bone marrow cellularity was seen. The authors postulate that the effects from the co-administration of phenol and hydroquinone in vivo are due to the increase in peroxidase-dependent metabolism of hydroquinone in the presence of phenol.

Current pharmacokinetic models focus on the liver as the organ primarily responsible for metabolizing benzene and pay little attention to the contribution made by the lung (Medinsky et al.,

1989; Travis et al., 1990). A recent study has shown that pharmacokinetic modeling which only takes into consideration metabolism by the liver underestimates the formation of phenol following benzene exposure (Sherwood and Sinclair, 1999). The authors suggest that other organs may be important in benzene metabolism. One possibility could be the lung. Rat pulmonary microsomes can readily metabolize benzene (Chaney and Carlson, 1995). Pulmonary metabolism of benzene may be important for several reasons. The lung is a target organ of benzene toxicity in animals (NTP, 1986; Snyder et al., 1988; Maltoni et al., 1989; Farris et al., 1993). The lungs receive 100% of the cardiac output. They are the first site of contact following inhalation.

The goals of this research were to determine the animal species most similar to human in benzene metabolism and to compare metabolism by hepatic and pulmonary microsomes at varying benzene concentrations. Male Sprague–Dawley rats were used because they develop an increased number of tumors following benzene exposure (Maltoni et al., 1989). They have also been used in several studies which examined various aspects of benzene metabolism (Snyder et al., 1993; Johansson and Ingelman-Sundberg, 1988), including investigations in this laboratory (Chaney and Carlson, 1995). Male NSA mice have demonstrated the ability to metabolize other xenobiotics dependent upon CYP2E1 including styrene (Carlson, 1997a,b), and *p*-nitrophenol (Carlson, 1997a), and therefore were used in this study. Male New Zealand White rabbits have also been used to study the metabolism of benzene (Johansson and Ingelman-Sundberg, 1988; Koop et al., 1989; Arinc et al., 1991) and were used in the study.

Concentrations of benzene used in these studies ranged from 24 to 1000 μM . The lower concentration was selected based on the studies of Schlosser et al. (1993) who concluded that the kinetics at the micromolar range were most relevant for the low dose exposures in humans. In their studies they used 4 μM benzene. The other concentrations in the experiments were chosen based on the studies of other investigators examining microsomal metabolism of benzene including Nakajima et

al. (1987) (0.0055 mM to 6.25 mM), Nakajima et al. (1985) (112 μM), Johansson and Ingelman-Sundberg (1988) (50 μM), and Koop et al. (1989) (2 mM). Inhalation studies in rats showed that following 2 h of exposure to 500 ppm of benzene, the blood concentration was $\sim 130 \mu\text{M}$ (Nakajima et al., 1985).

2. Materials and methods

2.1. Animals

Male New Zealand White rabbits (2–3 kg, Covance, Kalamazoo, MI), non-Swiss Albino (NSA) mice (20–25 g, Harlan Sprague–Dawley, Indianapolis, IN), and Sprague–Dawley rats (150–174 g, Harlan Sprague–Dawley, Indianapolis, IN) were used. Mice and rats were housed in group cages while rabbits were housed individually. Cages were kept in environmentally controlled rooms with a 12 h light:dark cycle. Mice and rats were given rodent laboratory chow (# 5001, Purina Mills, St. Louis, MO) and water ad libitum. Rabbits were given laboratory rabbit chow (HF # 5326, Purina Mills, St. Louis, MO) and water ad libitum.

2.2. Chemicals

Chemicals including L-ascorbic acid, NADH, and NADPH were obtained from Sigma (St. Louis, MO); magnesium chloride and potassium phosphate (monobasic) from Mallinckrodt Specialty Chemicals (Paris, KY); potassium chloride and potassium phosphate (dibasic) from J.T. Baker (Phillipsburg, NJ); and methanol from Fischer Scientific (Fairlawn, NJ). Bicinchoninic acid (BCA) protein assay kits were obtained from Pierce (Rockford, IL).

The internal standard contained benzene from MCB Reagents (Cincinnati, OH); butylated hydroxytoluene, hydroquinone, and catechol from Sigma (St. Louis, MO); ethyl acetate and 1,2,4-trihydroxybenzene from Aldrich (Milwaukee, WI); and phenol from Mallinckrodt Specialty Chemicals (Paris, KY).

[^{14}C]Benzene (specific activity = 52 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO).

2.3. Microsomal assays

A modification of the method developed by Chaney and Carlson (1995) was used for determining the microsomal metabolism of benzene. The animals were sacrificed, and liver and lung were removed. Samples of human tissues, collected during surgical procedures or at autopsy, were obtained from the Cooperative Human Tissue Network at The Ohio State University. Age, sex, and race of the human tissue donors are presented in Table 1. These samples were frozen shortly after removal and stored until use. Human tissues were processed the same way as tissues removed from the three laboratory species. Tissue was homogenized in potassium phosphate buffer (0.1 M, pH 7.4) containing 1.15% potassium chloride. This homogenate was centrifuged at $9000 \times g$ for 20 min. The supernatant was then centrifuged at $105\,000 \times g$ for 1 h to obtain the microsomal fraction. The pellet was resuspended in potassium phosphate buffer (0.1 M, pH 7.4). Part of this suspension was used for the microsomal assay while part was used to determine protein concentration using the bicinchoninic acid (BCA) assay.

Table 1
Human tissue donor information

Tissue	Age	Sex	Race
<i>Lung</i>			
H19	68	Female	White
H20	73	Male	NA ^a
H25	57	Female	White
H27	71	Male	NA
H29	67	Female	Black
<i>Liver</i>			
H23	78	Female	White
H24	47	Female	White
H26	30	Female	White
H28	37	Female	White
H30	81	Male	White

^a NA, information not available.

An incubation mixture containing microsomes ($\sim 3\text{--}4$ mg protein for liver and $\sim 0.2\text{--}1.2$ mg protein for lung), potassium phosphate buffer (0.1 M, pH 7.4) with ascorbate (0.007 M), magnesium chloride (2 mM), and [^{14}C]benzene (24, 200, 700, or 1000 μM) was placed in 3 ml Pierce (Rockland, IL) reacti-vials with resealable teflon septa and incubated at 37°C for 10 min. The reaction was started by injecting NADPH (1 mM) and NADH (1 mM), and the resulting mixture was incubated for 45 min.

The reaction was stopped by adding 0.5 ml of cold internal standard containing benzene (0.114 M), phenol (0.007 M), catechol (0.014 M), hydroquinone (0.026 M), 1,2,4-trihydroxybenzene (0.010 M), and butylated hydroxytoluene (0.091 M) in ethyl acetate. Extraction of the radiolabeled metabolites was maximized by vortexing the mixture, placing it on ice for 15 min, and repeating. The mixture was centrifuged at $833 \times g$ at 4°C for 10 min. A portion of the organic layer was removed, placed in a screw-top vial, and stored at -80°C until analysis.

2.4. Quantification of metabolites

The components of the organic phase were separated using a Beckman Model 332 HPLC System with a Beckman Ultrasphere octadecyl dodecyl sulfate (ODS) guard column (5 μm ; 4.6 mm \times 4.5 cm) and a Beckman ODS analytical column (5 μm ; 4.6 mm \times 25 cm). The mobile phases were deionized water purified by a Milli-Q Reagent Water System (A) and HPLC-grade methanol (B). Both phases were filtered through a 0.2 μm Nylaflo filter and degassed prior to use. Flow composition was as follows; 0–5 min: 100% A; 5–10 min: 80% A; 15–20 min: 50% A; 30–36 min: 100% B; 45–50: 100% A. Throughout the analysis the flow rate was 1 ml/min.

Following separation by HPLC the samples were analyzed using two different methods. Samples obtained prior to February 1998 were analyzed by a UV detector. Following UV analysis, eluent fractions were collected in 7 ml scintillation

Table 2

Benzene metabolism by mouse liver microsomes^a

Benzene (μM)	<i>n</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	9	1780 \pm 320 ^c	229 \pm 73 ^c	2750 \pm 430 ^c	0.65
200	6	2450 \pm 480 ^c	226 \pm 44 ^c	9900 \pm 1300 ^d	0.25
700	6	2710 \pm 820 ^c	257 \pm 96 ^c	16 800 \pm 2600 ^e	0.16
1000	10	3360 \pm 680 ^c	470 \pm 99 ^c	22 800 \pm 3500 ^e	0.15

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.^b Hydroquinone to phenol ratio.^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

vials containing 5 ml of Beckman Ready Safe scintillation cocktail (Beckman Instruments, Fullerton, CA). The contents of these vials were analyzed by a Beckman LS 3801 scintillation counter. The radioactive metabolites were identified by the time of their elution compared to the UV peaks of the internal standard. Samples obtained after February 1998 were analyzed by a UV detector to identify metabolites. Radioactivity was analyzed using a Packard 505TR Flow Scintillation Analyzer. Each ml of HPLC eluent mixed with 3 ml of Ultima Flo AP scintillation cocktail (Packard Instrument, Meriden, CT). The radioactive metabolites were identified by the time of their elution compared to the UV peaks of the internal standard. Metabolites were quantified as pmol metabolite/mg microsomal protein/45 min.

2.5. Statistical analysis

A minimum of six mice, six rats, three rabbits, or samples from three humans were used. For each individual species and tissue a one way ANOVA was performed to determine if any statistically significant difference existed for metabolite formation at various benzene concentrations. Student–Newman–Keuls' test was used to compare the means and determine which, if any, were significantly different. In order to normalize the data a logarithmic transformation was performed. For all of the tests the selected level of significance was 0.05.

3. Results

The concentration dependence of benzene metabolism was examined in mouse hepatic and pulmonary microsomal preparations. In microsomes from both organs phenol was the predominant metabolite followed by hydroquinone and catechol. In hepatic microsomes, hydroquinone and catechol formation were approximately doubled and phenol formation increased 8-fold over the concentration range of 24–1000 μM (Table 2). In pulmonary microsomes there was a much greater substrate concentration effect. This indicates that saturation of the enzyme system did not occur in lung but did in liver. Between the lowest and highest benzene concentrations, hydroquinone and catechol formation increased 33- and 40-fold, respectively (Table 3). Phenol formation increased by 17-fold. As the benzene concentration increased, the ratio of hydroquinone to phenol decreased in hepatic microsomes (Table 2). However, as the benzene concentration increased, this ratio increased in pulmonary microsomes (Table 3).

Metabolism of benzene by rabbit liver was similar to that by mouse liver (Table 4). In hepatic microsomes from rabbit, hydroquinone formation increased 12-fold, catechol 19-fold, and phenol 21-fold when the concentration of benzene was increased from 24 to 1000 μM (Table 4). As in the mouse, saturation of the enzyme system occurred in rabbit hepatic microsomes but not in pulmonary microsomes. In pulmonary microsomes, hydroquinone production increased 63-fold, cate-

chol 54-fold, and phenol production 141-fold (Table 5). In liver microsomes (Table 4), and in lung microsomes (Table 5), the hydroquinone to phenol ratio decreased as the benzene concentration was increased. The amount of phenol produced by lung approached that of liver at 1000 μM benzene.

In general, metabolism of benzene by rat was less than that in either mouse or rabbit (Tables 6 and 7). When the concentration of benzene was increased from 24 to 1000 μM in hepatic microsomal preparations, there was a 3-fold increase in hydroquinone formation and 6-fold increases in both catechol and phenol formation (Table 6).

Table 3

Benzene metabolism by mouse lung microsomes^a

Benzene (μM)	<i>n</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	8	65 \pm 18 ^c	12.8 \pm 2.4 ^c	370 \pm 100 ^c	0.18
200	6	422 \pm 34 ^d	77 \pm 20 ^c	1740 \pm 200 ^d	0.24
700	6	1220 \pm 300 ^c	169 \pm 64 ^c	4960 \pm 660 ^c	0.25
1000	8	2140 \pm 490 ^c	510 \pm 120 ^c	6200 \pm 1200 ^c	0.35

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.

^b Hydroquinone to phenol ratio.

^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

Table 4

Benzene metabolism by rabbit liver microsomes^a

Benzene (μM)	<i>N</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	3	298 \pm 21 ^c	22.2 \pm 1.9 ^c	683 \pm 85 ^c	0.44
200	3	1620 \pm 340 ^d	150 \pm 12 ^d	5030 \pm 980 ^d	0.32
700	3	3290 \pm 820 ^d	395 \pm 36 ^c	12 400 \pm 2500 ^c	0.26
1000	3	3590 \pm 940 ^d	423 \pm 33 ^c	14 600 \pm 2500 ^c	0.24

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.

^b Hydroquinone to phenol ratio.

^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

Table 5

Benzene metabolism by rabbit lung microsomes^a

Benzene (μM)	<i>N</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	3	10.8 \pm 3.6 ^c	0.99 \pm 0.94 ^c	96 \pm 11 ^c	0.11
200	3	91 \pm 17 ^d	6.4 \pm 3.5 ^d	1310 \pm 300 ^d	0.07
700	3	450 \pm 110 ^c	27.0 \pm 7.1 ^c	8700 \pm 2100 ^c	0.05
1000	3	680 \pm 150 ^c	53 \pm 18 ^c	13 500 \pm 3900 ^c	0.05

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.

^b Hydroquinone to phenol ratio.

^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

Table 6
Benzene metabolism by rat liver microsomes^a

Benzene (μM)	<i>n</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	6	135 \pm 14 ^c	10.1 \pm 0.9 ^c	1022 \pm 66 ^c	0.13
200	6	325 \pm 46 ^d	33.2 \pm 4.6 ^d	4040 \pm 410 ^d	0.08
700	6	424 \pm 52 ^d	51.8 \pm 6.8 ^d	5880 \pm 650 ^e	0.07
1000	6	388 \pm 30 ^d	58 \pm 10 ^d	5650 \pm 380 ^e	0.07

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.

^b Hydroquinone to phenol ratio.

^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

Table 7
Benzene metabolism by rat lung microsomes^a

Benzene (μM)	<i>N</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	6	7.5 \pm 0.6 ^c	0.7 \pm 0.4 ^c	34.9 \pm 5.4 ^c	0.21
200	6	69.3 \pm 8.4 ^d	13.3 \pm 6.6 ^c	258 \pm 22 ^d	0.27
700	6	274 \pm 41 ^e	36 \pm 19 ^c	849 \pm 47 ^e	0.32
1000	6	444 \pm 34 ^f	78 \pm 30 ^c	1190 \pm 120 ^e	0.37

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.

^b Hydroquinone to phenol ratio.

^{c,d,e,f} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

The dependence of metabolic activity on benzene concentration was greater in lung than in liver, just as was seen in mouse and rabbit. In pulmonary microsomes there was a 59-fold increase in the amount of hydroquinone formed, 111-fold increase in catechol, and a 34-fold increase in phenol formation (Table 7). Hepatic microsomes formed greater amounts of metabolites than pulmonary microsomes, except at the 1000 μM benzene concentration. At this concentration, pulmonary microsomes produced greater amounts of both hydroquinone and catechol than did microsomes from liver. As with mouse microsomes, the ratio of hydroquinone to phenol increased with an increase in benzene concentration for the lung (Table 7) but decreased for the liver (Table 6).

Human tissues demonstrated considerable benzene metabolizing activity which was highly dependent on the benzene concentration (Tables 8

and 9). When the concentration of benzene was increased from 24 to 1000 μM in human hepatic microsomes there was a 10-fold increase in hydroquinone, a 16-fold increase in catechol, and a 17-fold increase in phenol (Table 8). Pulmonary microsomes showed a 45-fold increase in hydroquinone formed and a 28-fold increase in phenol formation between the lowest and highest benzene concentrations (Table 9). Catechol was not consistently detected and was, therefore, omitted from the table. Although hepatic microsomes metabolized benzene to a greater extent than did pulmonary microsomes, pulmonary microsomes converted a greater proportion of phenol to hydroquinone as shown by the hydroquinone to phenol ratio (Table 9).

A summary of total metabolite formation and the hydroquinone:phenol ratio for each species at each concentration is presented in Table 10.

Table 8

Benzene metabolism by human liver microsomes^a

Benzene (μM)	<i>n</i>	Hydroquinone	Catechol	Phenol	HQ:P ^b
24	4	198 \pm 66 ^c	14 \pm 4 ^c	1460 \pm 350 ^c	0.14
200	5	940 \pm 240 ^d	80 \pm 17 ^d	9000 \pm 1100 ^d	0.10
700	3	1510 \pm 230 ^d	164 \pm 23 ^{d,e}	17 850 \pm 690 ^e	0.08
1000	5	1900 \pm 200 ^d	220 \pm 28 ^e	25 540 \pm 960 ^e	0.07

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.^b Hydroquinone to phenol ratio.^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

4. Discussion

A primary goal of this research was to compare benzene metabolism by three common laboratory species with that by humans. Rat and rabbit lungs were most similar to human in hydroquinone formation throughout the range of benzene concentrations studied. Mouse lung formed considerably greater amounts of this metabolite at all concentrations. In rabbit lung more phenol was formed than in other species. Therefore, except for catechol formation, which was not observed in human lung, rat is most similar to human.

Hepatic metabolism was more difficult to evaluate. At the lowest benzene concentration (24 μM), hepatic microsomes from rat and human were similar in the formation of all three metabolites. However, as the benzene concentration was increased, metabolism by human microsomes increased to a greater extent than that by rat microsomes. At 1000 μM benzene, hydroquinone and catechol formation by human liver was not quantitatively similar to any laboratory species, being higher than in rat but lower than in mouse or rabbit. However, phenol formation by human hepatic microsomes was found to be high and surprisingly similar to that of mouse.

The results are consistent with those of others. Orzechowski et al. (1995) demonstrated that hepatocytes from mice are better at metabolizing benzene than are those from rats. Brodfuehrer et al. (1990) showed that liver microsomes from mice formed greater amounts of phenol than those from humans which formed greater amounts than

those from rats. Schlosser et al. (1993) found that hepatic microsomes from mice metabolized a greater fraction of benzene than rats. In vivo experiments also show benzene is metabolized to a greater extent by mice than by rats (Henderson et al., 1989).

At the low benzene concentrations examined, rat is a good model for the oxidative metabolism of benzene in human lung and liver. Another important aspect of benzene metabolism related to its toxicity is detoxification via conjugation reactions. Conjugated metabolites of benzene include glutathione conjugates of benzene oxide, and sulfate and glucuronide conjugates of phenol, hydroquinone, catechol, and trihydroxybenzene (Henderson et al., 1989). A few studies have examined conjugation of benzene metabolites in the animal species used in the studies. Mouse hepatocytes are better at conjugating benzene metabo-

Table 9

Benzene metabolism by human lung microsomes^a

Benzene (μM)	<i>N</i>	Hydroquinone	Phenol	HQ:P ^b
24	5	14 \pm 4 ^c	37 \pm 11 ^c	0.38
200	5	128 \pm 32 ^d	290 \pm 75 ^d	0.44
700	3	370 \pm 120 ^d	790 \pm 170 ^e	0.47
1000	5	630 \pm 170 ^d	1030 \pm 170 ^e	0.61

^a Data are presented as mean \pm S.E. with units of pmols metabolite/mg protein/45 min.^b Hydroquinone to phenol ratio.^{c,d,e} Values with different superscripts are significantly different from one another ($P < 0.05$) for that metabolite.

Table 10
Summary of total metabolites^a and HQ:P^b

Species (μM)	Total metabolites		HQ:P	
	Liver	Lung	Liver	Lung
<i>Mouse</i>				
24	4759	448	0.65	0.18
200	12 576	2239	0.25	0.24
700	19 767	6349	0.16	0.25
1000	26 630	8850	0.15	0.35
<i>Rabbit</i>				
24	1003	108	0.44	0.11
200	6800	1407	0.32	0.07
700	16 085	9177	0.26	0.05
1000	18 613	14 233	0.24	0.05
<i>Rat</i>				
24	1167	43.1	0.13	0.21
200	4398	341	0.08	0.27
700	6356	1159	0.07	0.32
1000	6096	1712	0.07	0.37
<i>Human</i>				
24	1672	51	0.14	0.38
200	10 020	418	0.10	0.44
700	19 524	1160	0.08	0.47
1000	27 660	1660	0.07	0.61

^a Data are presented as means with units of pmols metabolite/mg protein/45 min.

^b Hydroquinone to phenol ratio.

lites than rat hepatocytes (Orzechowski et al., 1995). Henderson et al. (1989) showed that mice form greater amounts of conjugated benzene metabolites than rats in vivo. To fully evaluate the laboratory species most similar to human in benzene metabolism, the balance between oxidative metabolism and conjugation needs to be considered.

The second goal was to compare benzene metabolism by liver and lung microsomes. In the three laboratory species as well as in human, liver metabolized benzene to a greater extent than lung, with a few exceptions. Rabbit pulmonary microsomes formed nearly an equivalent amount of phenol as hepatic microsomes at 1000 μM benzene. With 1000 μM benzene rat pulmonary microsomes produced more hydroquinone and catechol than did hepatic microsomes. The enzyme system responsible for benzene metabolism

approached saturation in the hepatic microsomes for all three laboratory species and human, but this did not occur in pulmonary microsomes.

The results of this comparison show that although liver metabolizes more benzene than lung, lung still metabolizes a substantial amount of benzene. This finding may have important implications for pharmacokinetic modeling and ultimately in extrapolating animal data to human risk assessment. Current pharmacokinetic models do not take into consideration metabolism by lung (Medinsky et al., 1989; Travis et al., 1990). These models, which only take into consideration metabolism by liver, underestimate the formation of phenol following benzene exposure (Sherwood and Sinclair, 1999). The results show that benzene metabolism by lung is significant and needs to be included in pharmacokinetic models.

The hydroquinone to phenol ratios for mouse microsomes indicate that liver was more efficient at converting phenol to hydroquinone at 24 and 200 μM benzene, but lung was more efficient at the two higher benzene concentrations (Table 10). Pulmonary microsomes from both rat and human converted a greater proportion of phenol to hydroquinone than did hepatic microsomes. Conversely, in rabbit the hydroquinone to phenol ratio is greater for liver than for lung at all benzene concentrations examined, suggesting that liver is better at hydroxylating phenol.

In the three laboratory species and human the hydroquinone to phenol ratio varied with the benzene concentration. This may be due to competitive interactions between benzene and phenol for the catalytic site of CYP2E1 (Koop et al., 1989). Other researchers have shown that increasing the benzene concentration results in a decrease in the relative amount of hydroquinone formed versus phenol by liver (Koop et al., 1989; Kenyon et al., 1998) which is in agreement with the results in hepatic microsomes from all species as well as in rabbit pulmonary microsomes. However, the opposite effect was seen in pulmonary microsomes from mouse, rat, and human suggesting that lung metabolizes a higher percentage of the phenol as the benzene concentration is increased.

In summary, data from microsomal experiments indicate that rat is most similar to human

in oxidative pulmonary microsomal metabolism, except for catechol which was not found in human microsomes, at all benzene concentrations studied. In oxidative hepatic microsomal benzene metabolism rat is most similar to human at the lower concentrations, 24 and 200 μM , but at the higher benzene concentrations, 700 and 1000 μM , mouse is most similar to human in phenol formation. Overall, rat is most similar to human in oxidative benzene metabolism at the lower environmentally relevant levels in both liver and lung. In all species, the enzyme system responsible for benzene metabolism approached saturation in hepatic microsomes but not in pulmonary microsomes. The hydroquinone to phenol ratio decreased in the hepatic microsomes for all species, suggesting that hepatic microsomes have a greater affinity for benzene than phenol. The opposite effect occurred in pulmonary microsomes from all species except for rabbit. Several researchers have provided evidence for the competitive interactions of benzene and phenol (Koop et al., 1989; Kenyon et al., 1998). Considering that lung is the initial site of contact for inhaled benzene, receives 100% of the cardiac output, and has been found to be the site of tumor formation in animals exposed to benzene (NTP, 1986; Snyder et al., 1988; Maltoni et al., 1989; Farris et al., 1993), the results of the studies strengthen the argument that the lung plays an important role in benzene metabolism and, therefore, toxicity. The results also point out the importance of considering relevant benzene concentrations when measuring metabolic rates.

Acknowledgements

These studies were supported in part by grants from the National Institute of Health (ES04362), NIOSH TOI/CCTS 10467, and the United States Environmental Protection Agency. Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency through R826191 to Gary P. Carlson, it has not been subjected to the Agency's required peer review and therefore does not necessarily reflect the views of the Agency and

no official endorsement should be inferred. We are especially appreciative to the Midwestern Division of the Cooperative Human Tissue Network, located at The Ohio State University, for providing the human tissues.

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